Critical Review

The High Atherosclerotic Risk Among Epileptics: the Atheroprotective Role of Multivitamins

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Abstract. Neurologists have little concern about the high atherosclerotic risk among epileptics. Recent evidences mount that chronic epilepsy and prolonged use of antiepileptic drugs (AEDs) are associated with multiple risk factors that are critically implicated in pathobiology and dysfunction of the vessel wall through complex molecular mechanisms that promote atherogenesis. This review is concerned with three metabolic alterations, which are attributed as major risk factors for atherosclerosis among epileptics: altered metabolism of a) homocysteine (Hcy), b) lipids and lipoproteins, and c) uric acid. Most conventional AEDs reduce folic acid levels, thereby raising Hcy levels. Hyperhomosysteinemia is recently believed to induce endothelial dysfunction and promote atherosclerosis through complex oxidative and excitatory neurotoxic molecular mechanisms. However, Hcy itself is a convulsing substance with increased seizure recurrence and intractability to antiepileptic medications. AEDs can disturb lipid metabolism with resultant hypercholestrolemia and dyslipidemia, common recognized risks for atherosclerosis. Altered uric acid metabolism is common among epileptics. Uric acid has been implicated in endothelial cell damage and decreased endothelial nitric oxide bioavailability. In the presence of atherosclerotic milieu, uric acid interacts with other substrate toxicities and increased reactive oxygen species, accelerating atherosclerosis. The above information forms the rationale for future routine screening and correction of such metabolic alterations in epileptics. A convincing argument now develops that routine polyvitamin supplementation (folic acid, vitamin B12, vitamin B6, vitamin C, vitamin E, and β-carotene) becomes increasingly important for women and men receiving AEDs at all ages. The atheroprotective effect of multivitamins is through their antioxidant and anti-inflammatory effects together with their lipid and Hcy lowering effects.

Keywords: atherosclerosis, epilepsy, homocysteine, oxidative stress, multivitamin

Introduction

Epilepsy is a chronic dynamic medical problem that requires long-term and sometimes lifelong therapy. The tendency to have recurrent, unprovoked seizures occurs with a prevalence of about 0.5% and a cumulative lifetime prevalence of 3% (1). Many studies have demonstrated that prolonged treatment with antiepileptic drugs (AEDs) could have some undesirable metabolic side effects including elevated plasma concentration of homocysteine (Hcy) (2 – 4) and altered serum levels of cholesterol, lipoproteins (5, 6), and uric acid (7). These findings have important clinical relevance to vascular endothelial dysfunction and atherosclerosis-related diseases. The increasing recent interest is primarily because of the realization that various atherogenic stimuli, endothelial dysfunction, and oxidative stress are important for the complex nonadaptive inflammatory fibroproliferative changes collectively known as atherosclerosis (8, 9). Overproduction of reactive oxygen species (ROS) causes oxidation of low-density lipoproteins (LDLs) into atherogenic particles, which have
a pivotal role in the pathogenesis of atherosclerosis (10).

Hyperhomocysteinemia (HHcy), defined as Hcy level above 5 – 15 µmol/L (11, 12), has recently been commonly reported among epileptics (2 – 4). Many anticonvulsants reduce folic acid levels, thereby raising Hcy levels. Gene-drug interactions were suggested to induce convulsants reduce folic acid levels, thereby raising Hcy monly reported among epileptics (2 – 4). Many anti-

HHcy is recognized as important risk factors for atherosclerotic vascular diseases, independent of the long-recognized identified common risks (14, 15). The pathobioc hemical mechanism of arteriosclerosis and atherothrombosis in HHcy has not yet been elucidated. However, endothelial dysfunction has been proposed to result from the direct damaging effect of Hcy on the endothelium, at least in part, from altered oxidative status (16, 17). Autooxidation of Hcy promotes the production of hydroxyl radicals, thiolactone, and known lipid peroxidation initiators with creation of a pro-thrombotic environment (18 – 20). Additionally, studies demonstrated that folate deficiency was indeed capable of activating a redox-sensitive transcription factor, NF-κB, resulting from Hcy-mediated overproduction of hydrogen peroxide (H₂O₂) and ROS-mediated apoptosis (21).

Previous studies have demonstrated the influence of long-term antiepileptic therapy on the lipid profile of epileptics, particularly on apolipoprotein (Apo) serum levels (5, 6, 22). Hypercholesterolemia and dyslipidemia have long been known as important risks for atherosclerosis. Hypercholesterolemia is associated with increased endothelial permeability, retention of lipoproteins within the intima of the blood vessels, inflammatory cell recruitment, and formation of foam cells filled with oxidative-LDLs, which constitute by far the major part of the early fatty streak lesion. Such events play a significant role in the progression of fatty streaks to mature atherosclerotic plaque (23). A low level of high-density lipoprotein cholesterol (HDL-c) or dyslipidemia is also an important risk for vascular diseases and should be considered in determining treatment strategies (24, 25).

Hyperuricemia has recently been reported among epileptics (26). Experimental evidences suggest a complex but potentially direct causal role for uric acid in the pathogenesis of hypertension and atherosclerosis (27). Uric acid has been implicated in endothelial cell damage and decreased endothelial nitric oxide (eNO) bioavail-

Risk factors for atherosclerosis in epileptics

A) Hcy and epilepsy

Hcy is an essential thiol amino acid resulting from methylation of methionine, an essential amino acid derived from dietary proteins. Hcy is metabolized through two pathways: remethylation and transsulfuration, which use folic acid, vitamin B6, and vitamin B12 as cofactors. The genetic and acquired factors that induce a reduction in levels of folate, vitamin B6, and vitamin B12 cause an increase of p-Hcy levels (14). The relationship between Hcy and epilepsy will be discussed under three main topics: i) The excitatory neurotoxic and epileptogenic effect of Hcy, ii) The atherosclerotic risk of Hcy among epileptics, and iii) The teratogenic effect of Hcy.

1) The excitatory neurotoxic and epileptogenic effect of Hcy

The role of Hcy in vascular disease is well-supported (15, 31), but its direct excitatory neurotoxic effects has only recently been explored (32, 33). Early studies have demonstrated the genetic basis for homocystinuria as an inherited autosomal recessive metabolic disease with a worldwide distribution characterized by increased blood and brain Hcy levels caused by severe deficiency of cystathionine β-synthase activity (34), the enzyme catalyzing conversion of Hcy to cystathionine. Affected patients are usually presented with mental retardation, intractable seizures, atherosclerosis, ectopia lentis, and skeletal deformities. Interference with collagen cross-linking by sulphydryl groups of Hcy is the cause of ectopia lentis and skeletal deformities. Sulfation factor-like effects contribute to disruption of vascular endo-thelium, which is followed by platelet aggregation and widespread arterial and venous occlusion (34). Grieco (35) has demonstrated that deficiency of 5,10-MTHFR resulted in deranged vitamin B12 metabolism, leading to deficient remethylation of Hcy and low methionine homocystinuria.
The epileptogenic effect of severe HHcy (Hcy level: 50 – 200 µmol/L) is well-documented in experimental and human studies. Twenty percent of patients with severe HHcy has been reported to have seizures. However, this finding may not be generalizable to humans with mild and moderate HHcy (31, 36). Administration of high doses of Hcy has been found to induce seizures in immature and adult experimental animals with some age-dependent seizure pattern (37). Parenteral administration of Hcy has been found to activate experimental foci and produce focal seizures in experimental animals (38).

Electrocorticogram and generalized convulsions were visible in the recorded electrocorticogram and generalized convulsions in unlesioned animals (38).

The exact mechanism of the convulsant action of Hcy, both during development and in adulthood, remains to be clarified. It has been reported that Hcy induces neuronal cell death by stimulating N-methyl-D-aspartate (NMDA) receptors mediating excitotoxicity, as well as by producing free radicals (32, 39, 40) and induction of apoptosis (33). Its metabolites, homocysteic acid and l-Hcy sulphinic, also exhibit high excitotoxic potency by interaction with different glutamate receptor subtypes (41). In alcoholics, brain shrinkage is attributed to metabolic derangement associated with chronic alcoholism (e.g., ethanol-induced HHcy) with oxidative stress and excitotoxic neuronal damage (42). It has been demonstrated that combined treatment with low subthreshold doses of NMDA (AP7) and non-NMDA (NBQX) receptor antagonists resulted in pronounced anticonvulsant effect on the Hcy-induced seizures, which may be of potential significance for a new approach to epilepsy treatment (40).

Langmeier et al. (43) have demonstrated that the hippocampus is seriously affected by Hcy with prominent glial reaction in many regions and total loss of CA3 pyramidal cells. Dentate gyrus exhibiting marked changes in the CA1 cell layer with features of apoptotic cell death is seen in its granule cell layer. At the molecular level, exposure of rat hippocampal neurons to Hcy leads to activation of poly-ADP-ribose polymerase and NAD depletion, which precedes mitochondrial dysfunction, oxidative stress, caspase activation, and neuronal apoptosis (33). It has been suggested that stimulation of NMDA receptors by Hcy with excessive calcium influx causes reactive oxygen generation and neurotoxicity that contribute to the cognitive changes and the marked increased risk of cerebrovascular disease in children and young adults with homocystinuria. In addition, disruption of the blood-brain barrier in patients with stroke and HHcy exposes the brain to near plasma levels of Hcy and increases neurotoxicity (40).

ii) The atherosclerotic risk of Hcy among epileptics

Clinicians have paid relatively little attention to the potential atherosclerotic adverse event of AEDs. Phenytoin (PHT), carbamazepine (CBZ), and valproate (VPA) are still worldwide used AEDs, and they are predominantly utilized in developing countries (44, 45). Many prospective studies have demonstrated that prolonged treatment with anticonvulsants reduces folic acid levels, thereby raising Hcy levels and increasing the risk of vascular disease and cognitive impairment (2 – 4, 46, 47). Annegers et al. (48), for example, have demonstrated that younger adults (aged 25 to 64 years) with epilepsy and cerebrovascular disease have an increased mortality that is related to ischemic heart disease.

Various AEDs have differential effects on folic acid levels. Deficiency of folic acid with elevated Hcy levels appears prominent in epileptics receiving enzyme-inducing AEDs (2, 3), while in general, AEDs that do not induce cytochrome P450 enzymes are not associated with low folic acid levels (49). Many studies have demonstrated that the measurement of p-Hcy level at 6 h after a postmethionine load (PML) yields a higher sensitivity to detect HHcy in persons receiving CBZ, PHT, phenobarbital (PB), and primidone (4, 49). However, data on VPA effects on folic acid, an enzyme inhibitor AED, is conflicting. Most authors have reported that VPA does not reduce folate (50, 51), but may interfere with folate metabolism by inhibiting glutamate formyl transferase, an enzyme mediating the pathway that produces folic acid, a folic acid derivative (52).

Verrotti et al. (4) have reported that children on VPA have low folic acid and elevated Hcy levels. Lamotrigine (LTG) is an AED that has weak folic acid properties in vitro and has no effects on serum or red blood cell folate (53). Zonisamide (ZNS), a novel AED, is found to have no effect on folate level (50). Folate requirement is found to maintain a normal Hcy level in epileptics, especially homozygotes for the MTHFR 677 C → T mutation, receiving multiple AEDs (2, 13).

Plausible mechanisms through which HHcy can induce endothelial cell damage and dysfunction with increased risk for atherothrombotic and arteriosclerotic vascular disorders

The mechanisms by which Hcy induces atherosclerosis are largely unknown. Several biological mechanisms have been proposed to explain vascular pathological changes associated with HHcy including: a) Direct oxidative endothelial damage, b) Potentiation of LDL peroxidation, c) Malfunction of endothelium-dependent regulation of vascular tone and blood flow: endothelial nitric oxide synthase (eNOS) / nitric oxide
It has been shown that Hcy oxidizes LDLs by transduction pathways, resulting in oxidation of LDLs to interfere with many transcription factors and signal for endothelial dysfunction (64). Hcy has been found to decrease endothelial DNA synthesis and to alter DNA methylation and many regulatory proteins associated with the cell membrane (57). Hcy has been found to lower intracellular levels of adenosine, which has a cardio- and vaso-protective effect (58). Different experimental and in vitro studies have shown that the cytotoxic properties of Hcy can be ascribed to its generation of ROS in the presence of metal ions (iron and copper) and increase of lipid peroxidation (59). Formed H$_2$O$_2$ may also have direct harmful effects on the vascular endothelium (60). Malondialdehyde (MDA), a compound resulting from lipid peroxidation, has been found to have high ability to interact with lipoproteins. These modified lipoproteins are taken up by macrophages, which are transformed into foam cells that contribute to atherosclerotic plaque development and progression of atherogenesis (61). Another product of lipid peroxidation, thiobarbituric acid reaction substances (TBARS) has been found to induce oxidative damage through NMDA receptors, activation of nitric oxide synthase (NOS), and associated free radicals formation (62). Vascular NO production may prevent Hcy-induced endothelial dysfunction by S-nitrosylation (63).

Molecular human and animal cell culture studies have demonstrated that Hcy plays an important role in pathogenesis of atherosclerosis through induction of ROS, lipid peroxidation, up-regulation of p53-dependent Noxa expression, and mitochondrial cytochrome c release, which result in caspase-dependent endothelial cell apoptosis (63).

b) Potentiation of LDL peroxidation: During auto-oxidation of Hcy in plasma, ROS are generated. The latter initiates lipid peroxidation in circulating lipoproteins and cell membranes (potentially responsible for endothelial dysfunction) (64). Hcy has been found to interfere with many transcription factors and signal transduction pathways, resulting in oxidation of LDLs (64). It has been shown that Hcy oxidizes LDLs by reactions requiring redox-active transition of metal ions (10). Previous studies have demonstrated that the interaction between homocysteine-thiolactone (HcyT) and LDL induces the formation of homocystamide-LDL adducts (Hcy-LDL). Hcy-LDL exerts a cytotoxic effect through increasing lipid peroxidation and oxidative damage of endothelial cells. Structural and functional alterations of Hcy-LDL have been described and it has been suggested that homocysteinylination can increase atherogenicity of LDL (9).

Several experimental and human data confirmed the pro-oxidant and lipid peroxidation effect of HHCy: A high fasting plasma tHcy is found to be associated with enhanced lipid peroxidation as evidenced by quantifying lipid peroxidation markers, for example, plasma F$_2$-isoprostanes, prostaglandin-like products of nonenzymatic lipid peroxidation (8), MDA, unsaturated fatty acid concentrations (65), conjugated dienes, and TBARS (66). Hcy is found to significantly increase TBARS and decrease total radical-trapping antioxidant potential in rat hippocampus in a dose dependent manner (67).

High-methionine diet has been found to induce atherosclerosis in experimental models. A rat model of the acute methionine load-induced HHcy revealed that moderate HHcy plays a role in the development of a thrombogenic state, which is suggested to be mediated by lipid peroxidation (58, 68, 69). This has been confirmed by the presence of elevated plasma conjugated dienes, lipid hydroperoxides, and TBARS (69). Increased expression of TBARS following the oral methionine load test was also reported in human studies (69).

Moderate HHcy has been reported to be associated with normal aging, and this augments the oxidant effect on the endothelium and impairs intracellular antioxidant capacity, leading to enhanced lipid peroxidation and decreased total intracellular glutathione content (70).

c) Malfunction of endothelium-dependent regulation of vascular tone and blood flow: eNOS/NO system: Recently, NO and dysfunction of eNOS has been found to be closely implicated in the endothelial malfunction represented by impaired endothelium-dependent vasodilatory response in various vascular disorders including atherosclerosis (71). Hcy has been found to decrease the bioavailability of NO and impairs endothelial-dependent vasodilatation, and the latter can be reversed by administration of antioxidants (71–73). The pro-oxidant effect of Hcy appears to be blocked by the eNOS inhibitor L-$\text{N}^\text{G}$-nitroarginine methyl ester (74). In humans, experimental induction of HHcy by methionine loading was found to cause rapid and profound impairment of endothelium-dependent dilatation in both resistance and conduit arteries (73). eNOS was found to
become dysfunctional and produces superoxide rather than NO under conditions in which vascular tissue levels of tetrahydrobiopterin (BH4), a co-factor for eNOS, are deficient or lacking (71). Folates has been found to promote regeneration of BH4, which explains, at least in part, the observed restoration of the NO-generating capacity of the enzyme (75). In contrast, Upchurch et al. (76) have demonstrated that Hcy does not affect eNOS expression or its gene transcription, but it decreases the activity and steady state mRNA levels of glutathione peroxidase (GSH-Px), leading to enhanced generation of reduce oxygen intermediates.

d) Hcy promotes thrombosis and loss of endothelial cell antithrombotic function: Patients with HHcy have been found to be at increased risk for development of premature and recurrent arterial and venous thrombosis (77, 78). A substantial number of mechanisms has been proposed. Coagulation activation has been proposed as a mediator of p-Hcy-induced oxidative stress cell damage (79). In vitro cultured studies, utilizing extremely high doses of Hcy (1 – 10 µmol/L), in levels exceeding those encountered in severe pathological conditions, have demonstrated that Hcy induces non-specific inhibition of prostacyclin synthesis (60, 80), inhibition of protein C activation (81), activation of factor V (82), X, and XII (83), inhibition of natural anticoagulants (73), down-regulation of thrombocoealmodulin expression (84), increase platelet adhesion (80) due to impaired regulation of endothelium-derived relaxing factor and related nitrogen oxides (85), induction of tissue factor (86), suppression of heparan sulfate expression (87), and stimulation of smooth muscle proliferation (67). Hcy at 1 – 5 µmol/L has been found to specifically block tissue plasminogen activator, but not plasminogen binding to endothelial cells (88). Hcy concentrations as low as 8 µmol/L dramatically increase the affinity of lipoprotein(a) for plasmin-modified fibrin surfaces, thus inhibiting plasminogen activation. Lipoprotein(a) consists of LDL particles to which a highly glycosylated protein, Apo(a), is attached (87). Hcy also induces oxidation of LDLS in vitro (89). Oxidized LDL has been found to trigger platelet activation as well as induce some of the hemostatic abnormalities (90). In the study of Ebbesen et al. (91), whole blood coagulation profile monitoring (WBCP) that expresses quantitative and qualitative characteristics of the coagulation process has been carried out in a rat model with HHcy caused by folate deficiency (intermediate and severe HHcy). In this study, it was concluded that HHcy induces changes in dynamic time course of whole blood clotting through the following: a) it prolongs the initiation phase as evidenced by reduced turnover of thrombin with lower levels of thrombin-antithrombin complexes (91, 92) and increasing generation of thrombin (93). Increased inhibitor levels of tissue factor pathway could be another possible explanation of the prolonged initiation phase (94); b) it increases the maximum velocity of WBCP, which could be due to increased platelet activation and/or increased platelet aggregation (66, 95, 96); and c) it increases the maximum clot firmness as evidenced by thromboelastographic analysis (91). HHcy has been found to increase elastolysis, collagen accumulation, and fibrin synthesis and to modify fibrinogen in vivo, thereby causing an altered fibrin clot structure (acquired dysfibrinogenemia) and increasing its resistance to fibrinolysis, leading to increased risk of thrombosis (97). The Hcy thiol group has been proposed to affect the formation and structure of the plasmonic fibrin network (98). Hcy has been reported to promote the binding of lipoprotein(a) to fibrin (89). In the study of Lauricella et al. (98), clots formed from purified fibrinogen from Hcy-treated rabbits have been found to be composed of thinner fibers and can be lysed more slowly by plasmin than comparable clots from control fibrinogen. Scanning electronic microscopy also has shown that Hcy-associated networks are different from the control ones, with shorter, thicker, and more branched fibers, resulting in a more compact structure and probably more resistant to fibrinolysis.

e) Activation of inflammation: Association among B-vitamins deficiency, HHcy, oxidative stress, and enhanced activation of the cellular immune system has been found to exist in patients with immune-related diseases like some vascular diseases. It has been recognized that HHcy has been suggested to enhance the responsiveness of monocytes to inflammatory stimuli (99). Hcy causes the release of many inflammatory mediators that play an active role in atherosclerosis, such as interleukins, chemokines, tumor necrosis factor-α, and receptors for advanced glycation end-products and their signal-transducing ligands, resulting in increases in leukocyte recruitment into the atherosclerotic plaque and excessive adhesion of monocytes and neutrophils to endothelium (100).

iii) Hcy is a risk for teratogenicity

HHcy has been strongly implicated by many human and experimental studies in the teratogenic effects of anticonvulsants. Maternal HHcy is a well-established risk for embryonic toxicity and development of congenital defects, particularly dysmorphogenesis of the neural tube (exencephaly, cranioschisis, and spina bifida); neurocristopathies (increased the number of neural tube cells but decreased neural crest cell number); craniofacial defects; and cardiac defects (101). Early works have focused on the role of folate metabolism
because these defects are greatly reduced by folate supplementation (102, 103); however, more recent studies have confirmed the direct teratogenic effect of Hcy in presence of normal serum and erythrocyte folate (102, 104, 105). Rosenquist et al. (102) have demonstrated that avian embryos, in their early days, develop dysmorphogenesis when exposed directly to D,L-Hcy or with L-HcyT despite normal supplementary folate. Hcy has been suggested to disturb the developmental processes through numerous mechanisms as Hcy is included at the crossroads of many protein and DNA metabolisms (104–106). These mechanisms include a) direct chemical modification of proteins, b) generation of free radicals, c) perturbation of ligand binding to certain receptors, d) excessive apoptosis of embryonic nervous system cells, and e) perturbation of NMDA-like receptors or their precursors, which are expressed by early emigrating neural cells receptor. The American Academy of Neurology has recommended that all women during the childbearing period who are taking AEDs should consume at least 0.4 mg/day of folic acid (107).

B) Lipid and lipoprotein metabolism in epileptics

Differential lipid metabolism with AEDs

Increased risk is associated with the use of enzyme-inducer anticonvulsants as CBZ, PHT, and PB (22, 108, 109). Transient elevation in serum total cholesterol (TC), LDL-c, as well as triglycerides (TG) concentrations has been reported among patients receiving CBZ, PHT, and PB (5–7, 108, 109). High levels of HDL-c have been reported in normal subjects receiving PB (110). High serum levels of HDL-c, Apo-A, and Apo-B were reported with PB and CBZ (111). CBZ is associated with increase in HDL2 lipoprotein cholesterol, whereas PHT is specifically associated with increase of TG (112). In contrast, few studies reported that CBZ therapy does not affect serum lipids (TG, TC, HDL-c, LDL-c, and VLDL-c) (113). Lower values of TC, TG, HDL-c, and LDL-c similar to healthy controls have been found in epileptic patients receiving VPA, a well-known enzyme inhibitor (6, 110). Gender difference has been demonstrated with significant increase of HDL among women and higher LDL-c and TG but lower HDL-c in men that are independent to the dose or plasma concentrations of CBZ. These findings were suggested to be related to the diminished rate of death from coronary heart disease in patients with epilepsy, as HDL exerts an antiatherogenic effect (111).

The effect of AEDs on the serum level of lipids and lipoproteins could be explained on the basis of the different biotransformation pathways of the AEDs. CBZ, PHT, and PB are principally metabolized in the hepatic P450 system. This enzyme system also catalyzes the transformation of the cholesterol in biliary acids. Thus chronic treatment with these drugs might compete with cholesterol in the utilization of those enzymes, and this leads to reduction in the transformation of cholesterol in bile acids with increased TC level. Previous studies have suggested direct links running from the serum anticonvulsant levels to the extent of hepatic microsomal enzyme induction and further to the plasma HDL-c concentrations (114). On the other hand, the main route of VPA transformation in humans is represented by glucuronidation (115).

We have reported low serum level of HDL among epileptic patients receiving different AEDs as mono-therapy or in combination (unpublished data). We attribute this to: a) the influence of endogenous TG metabolism on HDL-c level. Epileptics with elevated TG or a combination of elevated TG and LDL-c have been found to have lower plasma HDL-c level than normolipidemic patients with epilepsy (116); b) the altered metabolism of the major HDL-Apo-A1 has been demonstrated in patients with abnormal levels of plasma HDL (117); and c) dietary factor is important for determination of an individualized lipid profile. High amount of fat in the diet has been reported to increase the conversion of HDL into LDL (118).

Although, the physiological determinants of HDL-c levels as a risk for atherosclerosis are poorly understood (23, 24), the protective effects of HDL-c have been attributed to its role in reverse cholesterol transport and its effects on endothelial cells. Several reports have suggested that HDL has anti-oxidative actions (24, 116).

The possible relationship among thyroid hormones balance and disturbed lipids and lipoproteins metabolism in epileptics

Relationship between AEDs and thyroid hormone balance

The suggestion that lipid abnormalities in epileptic patients are associated with subclinical thyroid disturbance remains controversial. The possible association between altered lipid metabolism and thyroid hormone balance in epileptics could be supported by the following: 1) Many previous studies have reported altered thyroid function (particularly low FT4), lipids, and lipoprotein metabolism among epileptics, particularly during treatment with enzyme inducer AEDs, CBZ, PHT, and PB (5, 6, 22–24, 108–114, 117, 119, 120). Previous studies have demonstrated that CBZ decreases the serum thyroid hormone levels, but thyrotropin (TSH) concentrations and TSH responses to TSH-releasing hormone remain normal. The reports on the effects of VPA on serum thyroid hormone were controversial.
(119, 120), 2) Thyroid hormones are well known to be involved in the regulation of lipoprotein metabolism, inducing significant changes in the concentration, size, and composition of plasma HDL (117, 121). In general, a higher prevalence of subclinical hypothyroidism in a population with hypercholesterolemia has been determined when compared to a population with normal cholesterol levels (122). Significant reductions in both TC and LDL-c concentrations have been reported after administration of thyroxine in a small group of hypercholesterolaemic patients with basal TSH levels in the upper range of normal values (123), 3) Many studies have confirmed that hypopituitarism is associated with deranged plasma lipid levels with lower HDL-c and higher TG level (124). 4) Previous and very recent studies have suggested that CBZ seems not to influence endogenous cholesterol synthesis or intestinal absorption directly, neither also relates to increased Apo-B production nor to decreased catabolism, but it changes the conversion cascade of intermediate-density lipoprotein particles by most likely an indirect effect through a decrease in thyroid hormones (119, 120, 125, 126).

However, it should be kept in mind that subclinical hypothyroidism is a relatively common condition with incidence between 3 to 7% in the general population with prevalence of approximately 20% (126, 127). Hence probably, such frequency rates for developing subclinical hypothyroidism might be increased among the epileptic group of the population, which is also common. Hamed et al. (128) in a very recent study analyzed thyroid hormones, lipid profile, and GGT in a group of 88 patients with epilepsy to determine whether thyroid dysfunction encountered in patients with epilepsy would also be associated with abnormal lipid profile. The authors concluded that a) altered lipid metabolism might be associated but not solely influenced by thyroid hormones, b) enzyme induction is not the main or only reason for altered thyroid function or HDL-c among patients with epilepsy. Hypothalamic/pituitary dysregulation by precisely mechanism caused by epilepsy itself or AEDs seems possible, and c) it is important to recognize that patients with epilepsy are at great risk for atherosclerosis, hence monitoring and correction of the culprit risks are mandatory.

Recent data indicate that subclinical hypothyroidism is also associated with enhanced risk for arteriosclerotic cardiovascular disease, which could be attributed to multiple mechanisms such as hyperlipidemia, hypercoagulable state, and direct effects on vascular smooth muscle or endothelial cells (127–129).

C) Uric acid metabolism in epileptics

Hyperuricemia has been recently reported among epileptics (untreated and treated) (7, 26). Despite controversies regarding uric acid being a risk factor for vascular diseases, uric acid has been implicated in vascular endothelial cell dysfunction. Unlike other risk factors associated with endothelial dysfunction, acute exposure to high concentrations of uric acid does not impair cardiovascular function in healthy men (130). However, it has been demonstrated that toxicities have a synergistic effect when acting in concert. In other words, antioxidants may become pro-oxidants in certain situations (28, 131, 132). Thus antioxidants may become pro-oxidants through what is known as the antioxidant-pro-oxidant urate redox shuttle which seems to rely heavily on its surrounding environment such as timing (early or late in the disease process), location within the tissue, acidity (acidic-basic-, or neutral PH), the surrounding oxidant milieu, depletion of other local antioxidants, the supply and duration of oxidant substrate and its oxidant enzyme, and uric acid levels (131–133). These individuals have abnormal elevations of uric acid of >6.5 or 7 mg/dL in men and >6.0 mg/dL in women, prooxidant (paradoxical conversion) state, while levels >4 mg/dL constitutes the normal physiologic and homeostatic range (134). In the early stages of the atherosclerotic process, uric acid is known to act as an antioxidant and may be one of the strongest determinants of plasma antioxidative capacity (132, 134). Later in the atherosclerotic process, the redox stress consumes the natural occurring local antioxidants such as superoxide dismutase (SOD), GPH-Px, and catalase. Uric acid can undergo the paradoxical antioxidant-prooxidant switch or the urate redox shuttle (132, 133, 135). In the rapidly progressing atherosclerotic vulnerable plaque, the intima of the vessel wall has been shown to be acidic (133), depleted of local antioxidants with an underlying increase in oxidant stress and ROS, and associated with uncoupling of the eNOS enzyme and a decrease in the locally produced naturally occurring antioxidant with endothelial cell damage within the microvascular bed at the capillary level within various affected organs. Additionally, LDLs such as LDL-c are capable of being modified and retained within the intima of the blood vessels through a process of oxidative modification by free radicals, hypochlorous acid, peroxynitrite, and selected oxidative enzymes such as xanthine oxidase, myeloperoxidase and lipoxygenase, and antioxidants (136, 137).

NO and vitamin C have each been shown to inhibit the prooxidant actions of uric acid during copper-mediated LDL-c oxidation (138). It is important to note...
that allopurinol and oxypurinol (XO inhibitors) are capable of reversing the impaired eNO production in both heart failure and type 2 diabetes mellitus (139, 140).

Relationship between Hcy and uric acid and atherosclerosis

A direct relation between Hcy and serum uric acid levels is known to occur in patients with atherosclerosis. Not only do these two track together (possibly reflecting an underlying elevated tension of redox stress), but also may be synergistic in creating an elevated tension of redox stress, especially in the rupture prone vulnerable atherosclerotic plaque with depletion of locally occurring antioxidants (141).

The atheroprotective effect of multivitamin supplementation

Clinical implications

Based on the above results, there will be a mandatory need for monitoring and treating elevated Hcy, fasting lipid profile (TC, HDL-c, LDL-c, and TG levels) and uric acid in epileptic patients. Recently, it has been demonstrated that the oxidative modification of LDLs in the endothelial microenvironment is a damaging factor that can be modified with fat-soluble antioxidant vitamins (46, 47, 142). Many prospective studies have demonstrated that a multi-ingredient vitamin formula (vitamin B6, vitamin B12, folate, vitamin C, vitamin E, and beta-carotene) with antioxidant properties has measurable effects on Hcy and LDL-c oxidation indices (29, 30). In the United States of America, there is a mandatory requirement that flour and cereals be fortified with 140 µg of folic acid per 100 g of flour, and it is estimated that this is associated with a 3% reduction in the risk of Hcy associated coronary artery stenosis (143).

Recently, elevation of the HDL-c levels has been considered as part of the patient’s overall profile of established risk factors in determining treatment strategies. It has been demonstrated that treatment with statins and/or with fibrates not only decrease cholesterol and LDL-c, but also produces an increase of HDL-c, which is very important in the regression of atherosclerosis (25, 144, 145). Administration of 500 mg vitamin C for more than 10 weeks significantly decreases TC and Apo-B in hyperlipidemias (146). It has been also demonstrated that co-supplementation of magnesium (200 mg/day), zinc (30 mg/day), and vitamins C (200 mg/day) and E (150 mg/day) based on their daily requirements significantly increases HDL-c and Apo-A1, but not TC, LDL-c, TG, and Apo-B (25). In addition to multi-vitamin supplementation, perhaps, prescribing a low-cholesterol diet may be needed in epileptic patients receiving AEDs, particularly enzyme inducers (22).

The anti-atherogenic protective mechanism of vitamins

Folic acid

It is well known that chronic use therapies to control seizures decreases serum folate level in half of epileptic patients, thus increasing the risk of folate depletion (2, 3, 11, 21, 29, 30, 46, 50, 52, 53, 91, 103, 107, 142, 147 – 149). Folate coenzymes are critical for de novo synthesis of purine and thymidine, and for interconversion of amino acids. Folate deficiency inhibits cellular proliferation, disturbs cell cycling, causes genetic damage, and eventually results in cell death (147). Folate deficiency is indeed capable of activating a redox-sensitive transcription factor, NF-kB, resulting form of the Hcy-mediated overproduction of H2O2, and this is crucial in the control of a ROS-mediated apoptosis (21). In addition to lowering the plasma levels of Hcy, low-dose folic acid treatment exerts beneficial effects on patients with HHcy by inhibiting pro-inflammatory responses such as chemokine secretion from human monocytes (99). The Hcy Lowering Trialists’ Collaboration (148) has concluded that folic acid at the doses between 0.5 and 5 mg/day lowers Hcy levels by 25%. A study comparing doses of 0.2, 0.4, 0.6, 0.8, and 1.0 mg of folic acid per day has demonstrated maximum Hcy reduction with the 0.8-mg dose (149). Based on this information, it seems prudent to ensure that men and women with epilepsy receiving AEDs, particularly enzyme-inducers, should receive adequate folic acid. For most individuals, this is best accomplished by giving a dietary supplement or prescription-strength folic acid tablets (1 mg each) or as part of a multivitamin supplements in which most contain 0.4 mg of folic acid (149).

However, attention should be paid to drug-vitamin interaction, since folic acid supplementations change the pharmacokinetics of some AEDs (150, 151). Folic acid has been found to increase the metabolism of PHT in a differential individualized manner (152, 153). Folic acid overload causes a considerable decrease in the hepatic and cerebral concentrations of PHT (154). Even in some studies, folic acid dosages as low as 1 mg/day perturbate PHT’s oxidative metabolism and this decrease could be responsible for the increased frequency of epileptic fits in patients treated with PHT (155). Hence, smaller deficiency preventive doses has been advised for PHT-treated patients with normal pretreatment folate levels (151). Some studies have demonstrated that folic acid supplementation should be initiated concomitantly each time with PHT therapy, for these reasons (150): a) to prevent decrease in folate.
and PHT and obtain steady-state concentrations sooner; b) to obtain better seizure control with no perturbation of PHT pharmacokinetics. It has been hypothesized that folate is a cofactor in PHT metabolism and may be responsible for the "pseudo-steady-state," which is a concentration where phenytoin appears to be at steady-state, but in reality, is not; and c) decrease adverse effects associated with folate deficiency.

**Vitamins B12 and B6**

The benefits of vitamins B12 and B6 on Hcy are more modest (156). Meta-analysis of the effect of vitamin B12 has suggested that a dose of 0.02 – 1 mg/day produces an additional reduction of Hcy by about 7%, but vitamin B6 has no additional effect (148). Patients with isolated methionine intolerance may benefit from vitamin B6 supplementation. (157).

**The antioxidant effect of micro- and macro-mineral**

Microminerals or trace elements, including zinc, selenium, and copper (Cu), are indispensable components for certain enzymes responsible for various metabolic processes in different tissues including the brain. They are important parts of antioxidant enzymes like SOD, GSH-Px, as well as transport protein with antioxidant properties, ceruloplasmin (Crl) (a Cu-binding protein). Trace elements are known to provide protection against peroxidative superoxide radicals damage. Through enzymatic or indirect action, trace elements can block destructive alteration of lipids, proteins, and nucleic acids by oxygen-derived free radicals, radiation, certain heavy metals, and other toxic substances. Vitamin E and GSH-Px have similar and complementary physiological roles in protecting cells from damage caused by endogenous peroxides (7). Many previous studies have demonstrated that the abnormal metabolism of trace elements, electrolytes, and antioxidants might be involved in the pathophysiology of severe mental and neurological disorders including epilepsy (7, 26). We and others (26, 158) have reported elevated levels of serum Cu and Crl in adult patients on chronic AED treatment, particularly enzyme inducers. During the last 2 decades, it has been proposed that atherogenesis involves multiple complex processes, among which is a complex interaction between atherogenic stimuli and micronutrients as Cu-Hcy complexes. Recently Cu-Hcy complexes have been suggested to actively participate in the biochemical responses of endothelial cells that are involved in the aethiopathogenesis of atherosclerosis (159). In the study of Apostolova et al. (159), Cu-Hcy complexes have been found to affect the metabolism of extracellular thiols more than Hcy alone and inhibit GSH-Px-1 activity and mRNA abundance. Cu-Hcy complexes are involved in remodeling and phosphorylation of focal adhesions and monocyte aggregation and diapedesis through endothelial cells.

Vitamin-trace element interaction should also be kept in mind during treating epileptic patients, particularly when the zinc requirement is known to increase as in children, adolescents, and pregnant, lactating, or elderly individuals. We and others have reported zinc deficiency in treated epileptic patients (26, 160). Some studies (161, 162) have raised the concern about the negative effect of folic acid supplementation on zinc absorption. Hansen et al. (163) have reported that inhibition of zinc absorption by folic acid is pronounced with low zinc meals than with high zinc meals. In contrast, others have reported that folic acid supplementation does not reduce plasma zinc concentrations (164). Hansen et al. (163) in his study, based on radioisotopic labeling and whole-body counting, have reported that the long-term effect of folic acid on zinc status per se is not previously evaluated effectively in humans because reliable biomarkers of zinc status are not available and zinc absorption studies will be more informative. The authors have concluded that the mechanism for the potential folate-zinc interaction could operate over the long-term, rather than at one moment in time.

**Conclusions**

Clearly, the potential comorbidity associated with a high Hcy level and altered lipid and uric acid metabolism, together with severe oxidative stress, in epileptics is significant. This information is essential for neurologists and should influence their current clinical practice as follow:

1) A mandatory need for monitoring serum or plasma levels of Hcy, fasting lipid profile (TC, HDL-c, LDL-c, TG), and lipoprotein and uric acid, particularly in patients on enzyme inducing AEDs.

2) Since serum Hcy levels are especially dependent on folate nutritional status, identifying patients with a genetic risk of HHcy and folate deficiency, homozygous for the C677T mutation, will be important. Those comprise 5% to 10% of the general population (165).

3) Measuring the fasting and PML p-Hcy level is necessary in women planning to get pregnant who are on enzyme inducing AEDs.

4) Attention should be paid to the elderly group of patients with seizures following a cerebrovascular accident treated with enzyme inducing AEDs (PHT, CBZ, and PB), since Hcy levels are more in such patients than in other age groups (14).

5) It seems prudent to ensure that men and women...
with epilepsy receiving AEDs, particularly enzyme-inducing AEDs should receive adequate multivitamin supplementation with combination of folic acid + vitamin B6 + vitamin B12 + β-carotene + vitamin C + vitamin E, taking into account the safety aspects of drug-vitamin and vitamin-nutrient interaction. Multivitamins have anti-inflammatory and antioxidant effect in addition to lipid and Hey lowing effect.

6) Considering the use of new AEDs (that do not induce hepatic enzymes) in patients at risk of HHcy or the prescription of combination therapy with folic acid + vitamin B6 + vitamin B12 to such patients as it has been shown to reverse HHcy (if the patients take only CBZ, then B12 should also be added).

7) Elimination of the basic well-known other risk factors of atherosclerosis is needed. In indicated cases, strict dietetic and pharmacological hypolipidemic treatment may be needed.

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